IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Amechand Boodhoo *et al.*	Group Art Unit: 1652
Serial No.: Not Yet Assigned	Examiner: Rebecca Prouty
Filed: herewith	
For: *Highly Purified Mocarhagin, A Cobra Venom Protease, Polynucleotides Encoding Same and Related Proteases, and Therapeutic Uses Thereof*	
Attorney Docket No.: GFN-5293CP2CN	

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CERTIFICATION UNDER 37 CFR 1.10

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I hereby certify that this 37 CFR 1.53(d) request and the documents referred to therein as enclosed are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Commissioner for Patents, U.S. Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202.

Ilidio P. Cardoso
Name of Person Mailing Paper

Signature of Person Mailing Paper

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination, please amend this application as follows:

In the Specification:

Please delete the first paragraph on page 1 and replace it with the following re-written paragraph:

-- This application is a continuation of application Serial No. 09/026,001, filed February 18, 1998, pending, which is a continuation-in-part of application Serial No. 09/012,637, filed January 23, 1998, now abandoned, which was a continuation-in-part of application Serial No. 08/843,373, filed April 15, 1997, now abandoned.--

Please amend the paragraph beginning at page 30, line 15 as follows:

-- Following SDS-PAGE and autoradiography, a novel ~50kD band appeared in the sample lane where 50 nanograms of purified bovine enterokinase had been inclubated with the conditioned medium (see Fig. 3). This band is consistent with the expected molecular weight of the mature protease when the propeptide (~23 kD) is cleaved off.--

In the Claims:

Please cancel claims 22-26, 32-36, 42-46, 52-56, 62-66, 72-76, 82-86, and 90-95 and amend claims 8, 13, 27, 37, 47, 57, 67, 77, and 87 as follows:

8. A method of treating an inflammatory disease comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

13. A method of treating an inflammatory disease comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

27. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

37. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

47. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

57. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

67. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

77. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

87. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:17 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

Please add new claims 96, 97, 98, and 99 as follows:

96. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

97. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

98. (New) The method of claims 96 or 97 wherein said condition characterized by P- or E-selectin mediated intercellular adhesion is selected from the group consisting of: myocardial infarction, vessel restenosis, thrombosis, bacterial or viral infection, metastatic conditions, inflammatory disorders such as arthritis, acute

respiratory distress syndrome, asthma, emphysema, delayed type hypersensitivity reaction, systemic lupus erythmatosus, thermal injury, autoimmune thyroiditis, experimental allergic encephalomyelitis, multiple sclerosis, diabetes, Reynaud's syndrome, neutrophilic dermatosis, inflammatory bowel disease, Grave's disease, glomerulonephritis, gingivitis, periodontitis, hemolytic uremic syndrome, ulcerative colitis, Crohn's decease, necrotizing enterocoltis, granulocyte transfusion associated syndrome, and cytokine-induced toxicity.

99. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of any one of claims 29, 39, 49, 59, 69, 79, or 89 to a mammalian subject.

REMARKS

Claims 1-95 are pending in the instant application. Claims 22-26, 32-36, 42-46, 52-56, 62-66, 72-76, 82-86, and 90-95 have been canceled. Claims 8, 13, 27, 37, 47, 57, 67, 77, and 87 have been amended and new claims 96, 97, 98, and 99 have been added. Accordingly, claims 1-21, 27-31, 37-41, 47-51, 57-61, 67-71, 77-81, and 87-89, and 96-99 are currently pending. For the Examiner's convenience, the pending claims are set forth in Appendix A.

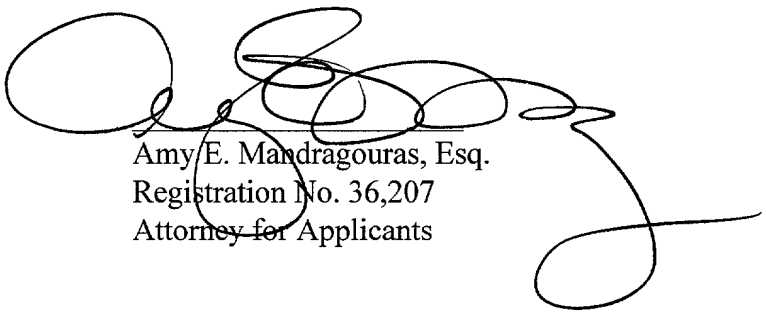
Support for the amendments to claims 8, 13, 27, 37, 47, 57, 67, 77, and 87 and new claims 96, 97, 98, and 99 may be found in the specification and claims as originally filed. In particular, support for new claims 96-99 may be found in the specification at page 18, line 23 through page 19, line 14. Applicants submit herewith a **"Version with Markings to Show Changes Made,"** which indicates the specific amendments made to the specification and the claims. *No new matter has been added.*

Any amendments to and/or cancellation of the claims should in no way be construed as an acquiescence to any of the Examiner's rejections and was done solely to expedite prosecution. Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

CONCLUSION

It is respectfully submitted that this application is in condition for allowance. If a telephone conversation with Applicants' attorney would help expedite the prosecution of the above-identified application, the Examiner is urged to call Applicants' attorney at (617) 227-7400.

Respectfully submitted,



Amy E. Mandragouras, Esq.
Registration No. 36,207
Attorney for Applicants

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, MA 02109
Tel. (617) 227-7400

Dated: November 27, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification

The first paragraph on page 1 has been replaced with the following re-written paragraph:

This application is a continuation of application Serial No. 09/026,001, filed February 18, 1998, pending, which is a continuation-in-part of application Serial No. 09/012,637, filed January 23, 1998, now abandoned, which was a continuation-in-part of application Serial No. 08/843,373, filed April 15, 1997, now abandoned.

The paragraph beginning at page 30, line 15 has been amended as follows:

Following SDS-PAGE and autoradiography, a novel ~50kD band appeared in the ~~smple~~ sample lane where 50 nanograms of purified bovine enterokinase had been inclubated with the conditioned medium (see Fig. 3). This band is consistent with the expected molecular weight of the mature protease when the propeptide (~23 kD) is cleaved off.

In the Claims:

Claims 22-26, 32-36, 42-46, 52-56, 62-66, 72-76, 82-86, and 90-95 have been cancelled.

Claims 8, 13, 27, 37, 47, 57, 67, 77, and 87 have been amended as follows:

8. A method of treating an inflammatory disease ~~which comprises~~ comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

13. A method of treating an inflammatory disease ~~which comprises~~ comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

27. A protein produced according to ~~the process of claim 26~~ a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

37. A protein produced according to ~~the process of claim 26~~ a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

47. A protein produced according to ~~the process of claim 26~~ a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

57. A protein produced according to ~~the process of claim 26~~ a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

67. A protein produced according to ~~the process of claim 26~~ a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

77. A protein produced according to ~~the process of claim 26~~ a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

87. A protein produced according to ~~the process of claim 26~~ a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:17 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

New claims 96, 97, 98, and 99, have been added as follows:

96. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

97. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

98. (New) The method of claims 96 or 97 wherein said condition characterized by P- or E-selectin mediated intercellular adhesion is selected from the group consisting of: myocardial infarction, vessel restenosis, thrombosis, bacterial or viral infection, metastatic conditions, inflammatory disorders such as arthritis, acute respiratory distress syndrome, asthma, emphysema, delayed type hypersensitivity reaction, systemic lupus erythmatosus, thermal injury, autoimmune thyroiditis, experimental allergic encephalomyelitis, multiple sclerosis, diabetes, Reynaud's syndrome, neutrophilic dermatosis, inflammatory bowel disease, Grave's disease, glomerulonephritis, gingivitis, periodontitis, hemolytic uremic syndrome, ulcerative colitis, Crohn's decease, necrotizing enterocoltis, granulocyte transfusion associated syndrome, and cytokine-induced toxicity.

99. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of any one of claims 29, 39, 49, 59, 69, 79, or 89 to a mammalian subject.

APPENDIX A

1. A composition comprising a mocarhagin protein at least 95% free of other cobra proteins.

2. The composition of claim 1 wherein said mocarhagin protein is full-length mocarhagin.

3. The composition of claim 1 wherein said mocarhagin protein is a fragment of full-length mocarhagin having mocarhagin proteolytic activity.

4. The composition of claim 1 wherein said mocarhagin protein exhibits an IC_{50} of less than about 100 μg/mL in a neutrophil/HL60 binding inhibition assay.

5. The composition of claim 1 wherein said mocarhagin protein is characterized by at least one characteristic selected from the group consisting of:

(a) a molecular weight of approximately 55 kDa under reducing conditions;

(b) a molecular weight of approximately 55 kDa under nonreducing conditions;

(c) an N-terminal amino acid sequence comprising

TNTPEQDRYLQAKKYIEFYVVVDNVMYRKY (SEQ NO 1);

(d) mocarhagin proteolytic activity;

(e) the ability to inhibit platelet binding to vWF;

(f) requirement of calcium ion for activity;

(g) requirement of zinc ion for activity

(h) an activity substantially inhibited by excess EDTA; and

(i) an activity substantially inhibited by high concentrations of DFP.

6. The composition of claim 1 wherein said mocarhagin protein is capable of cleaving a material selected from the group consisting of anionic polypeptides containing sulfated tryosine residues, PSGL-1 and GP1bα.

7. A composition comprising a therapeutically effective amount of a composition of claim 1 and a pharmaceutically acceptable carrier.

8. A method of treating an inflammatory disease comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

9. A method of inhibiting selecting-mediated binding comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

10. A method of isolating mocarhagin from venom, said method comprising:

(a) subjecting a composition comprising cobra venom to a heparin affinity chromatography column;

(b) subjecting the elute from said heparin affinity column to a size exclusion column;

(c) subjecting the eluate from said size exclusion column to a Mono S column; and

(d) eluting said mocarhagin from said Mono S column.

11. A composition comprising a protein isolated according to the method of claim 10.

12. The composition of claim 11 further comprising a pharmaceutically acceptable carrier.

13. A method of treating an inflammatory disease comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

14. A method of inhibiting selectin-mediated binding comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

15. A composition comprising an antibody which specifically reacts with the mocarhagin of the composition of claim 1 or a fragment thereof having mocarhagin proteolytic activity.

16. The composition of claim 4 wherein said mocarhagin protein exhibits of IC_{50} of less than about 1 μg/mL in a neutrophil/HL60 binding inhibition assay.

17. The composition of claim 1 wherein said mocarhagin protein is homogeneous.

18. The composition of claim 1 wherein the N-terminal sequence of said protein is

TNTPEQDRYLQAKKYIEFYVVVDNVMYRKYTGKLHVITXXVYEMNALN (SEQ ID NO: 2).

19. The composition of claim 5 wherein said protein comprises the amino acid sequence of SEQ ID NO: 6 from amino acid 192 to amino acid 621.

20. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO: 6;

(b) the amino acid sequence of SEQ ID NO: 6 from amino acid 24 to amino acid 621;

(c) the amino acid sequence of SEQ ID NO:6 from amino acid 192 to amino acid 621;

(d) fragments of the amino acid sequence of SEQ ID NO: 6 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-1 deposited under accession number ATCC 209588;

the protein being substantially free from other mammalian proteins.

21. The composition of claim 20 wherein said protein comprises the amino acid sequence of SEQ ID NO: 6.

27. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:5 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

28. The protein of claim 27 comprising a mature protein.

29. A pharmaceutical composition comprising a protein of claim 20 and a pharmaceutically acceptable carrier.

30. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:8;

(b) the amino acid sequence of SEQ ID NO:8 from amino acid 24 to amino acid 439;

(c) the amino acid sequence of SEQ ID NO: 8 from amino acid 192 to amino acid 439;

(d) fragments of the amino acid sequence of SEQ ID NO:8 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-2 deposited under accession number ATCC 209589;

the protein being substantially free from other mammalian proteins.

31. The composition of claim 30 wherein said protein comprises the amino acid sequence of SEQ ID NO: 6.

37. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:7 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

38. The protein of claim 37 comprising a mature protein.

39. A pharmaceutical composition comprising a protein of claim 30 and a pharmaceutically acceptable carrier.

40. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO: 10;

(b) the amino acid sequence of SEQ ID NO: 10 from amino acid 24 to amino acid 613;

(c) the amino acid sequence of SEQ ID NO: 10 from amino acid 192 to amino acid 613;

(d) fragments of the amino acid sequence of SEQ ID NO:10 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-9 deposited under accession number ATCC 209586;

the protein being substantially free from other mammalian proteins.

41. The composition of claim 40 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

47. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:9 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

48. The protein of claim 47 comprising a mature protein.

49. A pharmaceutical composition comprising a protein of claim 40 and a pharmaceutically acceptable carrier.

50. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO: 12;

(b) the amino acid sequence of SEQ ID NO: 12 from the amino acid 24 to amino acid 521;

(c) the amino acid sequence of SEQ ID NO:12 from amino acid 192 to amino acid 521;

(d) fragments of the amino acid sequence of SEQ ID NO:12 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-12 deposited under accession number ATCC 209585;

the protein being substantially free from other mammalian proteins.

51. The composition of claim 50 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

57. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:11 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

58. The protein of claim 57 comprising a mature protein.

59. A pharmaceutical composition comprising a protein of claim 50 and a pharmaceutically acceptable carrier.

60. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:14;

(b) the amino acid sequence of SEQ ID NO: 14 from amino acid 24 to amino acid 592;

(c) the amino acid sequence of SEQ ID NO: 14 from amino acid 192 to amino acid 592;

(d) fragments of the amino acid sequence of SEQ ID NO:12 encoding a protein having mocarhagin activity; and

(e) the amino acid sequence encoded by the cDNA insert of clone NMM-13 deposited under accession number ATCC 209584;

the protein being substantially free from other mammalian proteins.

61. The composition of claim 60 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

67. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

68. The protein of claim 67 comprising a mature protein.

69. A pharmaceutical composition comprising a protein of claim 60 and a pharmaceutically acceptable carrier.

70. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO: 16;

(b) the amino acid sequence of SEQ ID NO: 16 from amino acid 62 to amino acid 462;

(c) fragments of the amino acid sequence of SEQ ID NO: 16 encoding a protein having mocarhagin activity; and

(d) the amino acid sequence encoded by the cDNA insert of clone NMM-3 deposited under accession number ATCC 209587;

the protein being substantially free from other mammalian proteins.

71. The composition of claim 70 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

77. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

78. The protein of claim 77 comprising a mature protein.

79. A pharmaceutical composition comprising a protein of claim 70 and a pharmaceutically acceptable carrier.

80. A composition comprising a mocarhagin protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO: 18;

(b) the amino acid sequence of SEQ II) NO: 18 from amino acid 197 to amino acid 621;

(c) fragments of the amino acid sequence of SEQ ID NO:18 encoding a protein having mocarhagin activity; and

(d) the amino acid sequence encoded by the cDNA insert of clone NMM-9ek deposited under accession number ATCC 209583;

the protein being substantially free from other mammalian proteins.

81. The composition of claim 80 wherein said protein comprises the amino acid sequence of SEQ ID NO:6.

87. A protein produced according to a process comprising:

(a) in a suitable culture medium, growing a culture a host cell transformed with an isolated polynucleotide comprising the nucleotide sequence of SEQ ID NO:17 operably linked to an expression control sequence; and

(b) purifying the protein from the culture.

88. The protein of claim 87 comprising a mature protein.

89. A pharmaceutical composition comprising a protein of claim 80 and a pharmaceutically acceptable carrier.

96. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of claim 7 to a mammalian subject.

97. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective amount of a composition of claim 12 to a mammalian subject.

98. (New) The method of claims 96 or 97 wherein said condition characterized by P- or E-selectin mediated intercellular adhesion is selected from the group consisting of: myocardial infarction, vessel restenosis, thrombosis, bacterial or viral infection, metastatic conditions, inflammatory disorders such as arthritis, acute respiratory distress syndrome, asthma, emphysema, delayed type hypersensitivity reaction, systemic lupus erythmatosus, thermal injury, autoimmune thyroiditis, experimental allergic encephalomyelitis, multiple sclerosis, diabetes, Reynaud's syndrome, neutrophilic dermatosis, inflammatory bowel disease, Grave's disease, glomerulonephritis, gingivitis, periodontitis, hemolytic uremic syndrome, ulcerative colitis, Crohn's decease, necrotizing enterocoltis, granulocyte transfusion associated syndrome, and cytokine-induced toxicity.

99. (New) A method of treating a condition characterized by P- or E-selectin mediated intercellular adhesion comprising administering a therapeutically effective

amount of a composition of any one of claims 29, 39, 49, 59, 69, 79, or 89 to a mammalian subject.